conclusion, 14q32 microRNA inhibition may offer an alternative to growth factors in therapeutic neovascularization.

Epigenetic Modifications in the Development of Atherosclerosis

J. Pelisek, A. Greißel, M. Culmes, A. Zernecke, H. Eckstein

Department of Vascular and Endovascular Surgery, Klinikum rechts der Isar der Technische Universitaet Muenchen, Germany

Introduction: Epigenetics is playing a decisive role in the regulation of gene expression. Furthermore, pathological epigenetic changes have already been associated with various diseases, especially cancer. However, few data are so far available about epigenetic modifications in atherosclerotic lesions. Therefore, we investigated histone methylation and acetylation in different stages of atherosclerosis in patients with carotid artery stenosis.
Methods: Carotid plaques ($n = 120$) from our biobank were classified histologically according to AHA and divided as early (type I—III, $n = 60$) or advanced (type V—VII, $n = 60$) stage of atherosclerosis. Twelve healthy vessels served as controls. Expression of histone methyl and acetyltransferases were analysed by SYBRgreen-based real-time RT-PCR. Immunohistochemistry and western blotting were performed to quantify expression at protein level and to associate histone methylation with the individual cells within the atherosclerotic plaques.
Results: In atherosclerosis, methylation of H3K4 was unaltered. In contrast methylation of H3K9 and H3K27 were significantly decreased, compared to control arteries. Expression of H3K4 was increased and H3K9 decreased in smooth muscles, whereas H3K9 and H3K27 were reduced in inflammatory cells in advanced versus early atherosclerosis. MML2 and G9a significantly increased in advanced versus early atherosclerosis, and a significant decrease in expression of G9a was observed between controls and early stages. Increased histone acetylation was observed on H3K9 and H3K27 in SMCs in advanced atherosclerotic lesions compared to healthy vessels, acetylation of H3 at position K9 in SMCs and macrophages was associated with plaque progression. Expression of acetyltransferase GCN5L and MYST1 correlated with severity of atherosclerosis.
Conclusion: The extent of histone methylation and acetylation, as well the expression of corresponding transferases were significantly altered in atherosclerotic lesions and were significantly associated with the progression of atherosclerosis, thus suggesting an important contribution of epigenetic mechanisms to plaque progression and vulnerability.

Dysfunctional Endothelial Progenitor Cells may Contribute to Vasculopathy in Systemic Sclerosis

R.W. Good, S.L. Trinder, C.P. Denton, D.J. Abraham, A.M. Holmes

Rheumatology and Connective Tissue Diseases, UCL Medical School, University College London, UK

Introduction: Vascular complications associated with systemic sclerosis (SSc) including pulmonary arterial hypertension (PAH-SSc), result from endothelial damage and loss of barrier function. The causes of endothelial dysfunction are unclear, but endothelial integrity is likely to be significantly diminished in SSc. Endothelial progenitor cells (EPCs) derived from peripheral blood mononuclear cells (PBMCs) express endothelial and haematopoietic markers. It is thought they home to sites of vascular injury and differentiate into endothelial cells and restore the barrier. In SSc patients circulating levels of EPCs are reduced. This study aimed to: (i) Develop a robust method to isolate, grow and characterise healthy control (HC) and SSc EPCs from PBMCs. (ii) Compare the cellular functions of EPCs to mature endothelial cells. (iii) Elucidate the functional role of EPCs in SSc vasculopathy.
Methods: Peripheral blood was taken from HC ($n = 10$) and SSc donors ($n = 10$). EPCs were cultured from PBMCs. EPCs were characterised by flow cytometry, then EPCs and human pulmonary artery endothelial cells (hPAECs) were seeded into transwell inserts and grown to confluence. Cells were incubated with TNFα (10 ng/ml), and their capacity to form biological barriers and support immune cell influx was assessed using FITC-albumin (0.5 mg/ml) and neutrophil transmigration. We further assessed the responses of EPCs to TNFα stimulation by ELISA to quantify pro-inflammatory cytokine release.
Results: We demonstrate that EPCs, characterised by flow cytometry to be CD34+/CD31+/VEGFR2+/CD105+ and CD133+, can form biological barriers with similar capabilities as mature hPAECs in vitro. TNFα significantly enhanced permeability of EPCs ($P < 0.05$) and hPAECs ($P < 0.05$) monolayers. Mixed cultures of 10% SSc-EPCs and 90% mature PAECs significantly enhanced endothelial permeability compared to HC-EPC/PAECs mixed cultures. Consistent with EPCs possessing similar cellular activities as mature endothelial cells, TNFα stimulated neutrophil transmigration in monolayers of EPCs ($P < 0.05$) and hPAECs ($P < 0.05$) and enhanced the secretion of IL-8 in both EPCs ($P < 0.01$) and hPAECs ($P < 0.05$).
Conclusion: We have demonstrated that endothelial progenitors can maintain an endothelial barrier consistent with that observed by mature hPAECs in vitro; established that endothelial barriers consisting of both EPCs and mature endothelial cells are dysfunctional in SSc; and finally, shown that EPCs respond to TNFa in a similar manner to mature PAECs, secreting pro-inflammatory cytokines such as IL-8 and supporting neutrophil transmigration. The biological function and importance of EPCs from SSc patients in vasculopathy, restoration and maintenance of the endothelial barrier function remains unclear.

The Role of Erythropoietin in Skeletal Muscle Ischaemia In Vitro and In Vivo

R.S. Yu, D. Baker, S. Kolvekar, D. Abraham, J.C.S. Tsui

University College London, UK